

WHAT IS CLAIMED IS:

1                   1.       An isolated infectious chimeric parainfluenza virus (PIV) comprising  
2 a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase  
3 protein (L), and a partial or complete PIV vector genome or antigenome combined with one  
4 or more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
5 determinant(s) of one or more heterologous pathogen(s) to form a chimeric PIV genome or  
6 antigenome.

1                   2.       The chimeric PIV of claim 1, wherein said one or more heterologous  
2 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added as  
3 supernumerary gene(s) or genome segment(s) adjacent to or within a noncoding region of  
4 the partial or complete PIV vector genome or antigenome.

1                   3.       The chimeric PIV of claim 1, wherein said one or more heterologous  
2 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for  
3 one or more counterpart gene(s) or genome segment(s) in a partial PIV vector genome or  
4 antigenome.

1                   4.       The chimeric PIV of claim 1, wherein said one or more heterologous  
2 pathogens is a heterologous PIV and said heterologous gene(s) or genome segment(s)  
3 encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or fragment(s)  
4 thereof.

1                   5.       The chimeric PIV of claim 1, wherein the vector genome or  
2 antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the  
3 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of  
4 one or more heterologous PIV(s).

1                   6.       The chimeric PIV of claim 5, wherein said one or more heterologous  
2 PIV(s) is/are selected from HPIV1, HPIV2, or HPIV3.

1                   7.       The chimeric PIV of claim 5, wherein the vector genome or  
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 heterologous HPIV(s).

1                   8.       The chimeric PIV of claim 7, wherein the vector genome or  
2 antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 heterologous HPIV(s).

1                   9.       The chimeric PIV of claim 8, wherein one or more gene(s) or genome  
2 segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from HPIV1  
3 HN and F glycoproteins and antigenic domains, fragments and epitopes thereof is/are added  
4 to or substituted within the partial or complete HPIV3 genome or antigenome.

1                   10.      The chimeric PIV of claim 8, wherein the vector genome or  
2 antigenome is a partial or complete HPIV3 JS genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 heterologous HPIV(s).

1                   11.      The chimeric PIV of claim 10, wherein one or more gene(s) or  
2 genome segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from  
3 HPIV1 HN and F glycoproteins and antigenic domains, fragments and epitopes thereof is/are  
4 added to or substituted within the partial or complete HPIV3 JS genome or antigenome.

1                   12.      The chimeric PIV of claim 9, wherein both HPIV1 genes encoding  
2 HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes in a partial  
3 HPIV3 vector genome or antigenome.

1                   13.      The chimeric PIV of claim 9, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 present within PIV3 JS *cp45* selected from mutations specifying an amino acid substitution  
4 in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in  
5 the N protein at a position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C  
6 protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide substitution a 3' leader  
7 sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of  
8 JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to  
9 nucleotide 62 of JS *cp45*



22. The chimeric PIV of claim 1, wherein the vector genome or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

23. The chimeric PIV of claim 22, wherein said one or more heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus pre M, E, and NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

1                    24.      The chimeric PIV of claim 22, wherein the vector genome or  
2      antigenome is a partial or complete HPIV3 genome or antigenome or a chimeric HPIV  
3      genome or antigenome comprising a partial or complete HPIV3 genome or antigenome  
4      having one or more gene(s) or genome segment(s) encoding one or more antigenic  
5      determinant(s) of a heterologous HPIV added or incorporated therein.

1                    25.     The chimeric PIV of claim 24, wherein the heterologous pathogen is  
2     measles virus and the heterologous antigenic determinant(s) is/are selected from the measles  
3     virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1                    26.     The chimeric PIV of claim 25, wherein a transcription unit comprising  
2     an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within  
3     a HPIV3 vector genome or antigenome.

1                    27.     The chimeric PIV of claim 26 selected from rPIV3(HA HN-L),  
2     rPIV3(HA N-P), *rcp45L*(HA N-P), rPIV3(HA P-M), or *rcp45L*(HA P-M).

1           28.     The chimeric PIV of claim 24, wherein the vector genome or  
2 antigenome is a chimeric HPIV genome or antigenome comprising a partial or complete  
3 HPIV3 genome or antigenome having one or more gene(s) or genome segment(s) encoding  
4 one or more antigenic determinant(s) of HPIV1 added or incorporated therein.

1           29.     The chimeric PIV of claim 25, wherein the heterologous pathogen is  
2 measles virus and the heterologous antigenic determinant(s) is/are selected from the measles  
3 virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1           30.     The chimeric PIV of claim 29, wherein a transcription unit comprising  
2 an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within  
3 a HPIV3-1 vector genome or antigenome having both the HPIV3 HN and F ORFs  
4 substituted by the HN and F ORFs of HPIV1.

1           31.     The chimeric PIV of claim 30, selected from rPIV3-1 HA<sub>P-M</sub> or  
2 rPIV3-1 HA<sub>P-M</sub> *cp45L*.

1           32.     The chimeric PIV of claim 1, wherein the partial or complete PIV  
2 vector genome or antigenome is combined with one or more supernumerary heterologous  
3 gene(s) or genome segment(s) to form the chimeric PIV genome or antigenome.

1           33.     The chimeric PIV of claim 32, wherein the vector genome or  
2 antigenome is a partial or complete HPIV3 genome or antigenome and said one or more  
3 supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN,  
4 HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene  
5 unit.

1           34.     The chimeric PIV of claim 33, wherein one or both of the HPIV1 HN  
2 and/or HPIV2 HN ORF(s) is/are inserted within the HPIV3 vector genome or antigenome,  
3 respectively.

1           35.     The chimeric PIV of claim 33, wherein the HPIV1 HN, HPIV2 HN,  
2 and measles virus HA ORFs are inserted between the N/P, P/M, and HN/L genes,  
3 respectively.

36. The chimeric PIV of claim 33, wherein the HPIV1 HN and HPIV2 HN genes are inserted between the N/P and P/M genes, respectively and a 3918-nt GU insert is added between the HN and L genes.

37. The chimeric PIV of claim 33, which is selected from rHPIV3 1HN<sub>N-P</sub>, rHPIV3 1HN<sub>P-M</sub>, rHPIV3 2HN<sub>N-P</sub>, rHPIV3 2HN<sub>P-M</sub>, rHPIV3 1HN<sub>N-P</sub> 2HN<sub>P-M</sub>, rHPIV3 1HN<sub>N-P</sub> 2HN<sub>P-M</sub> HA<sub>HN-L</sub>, and rHPIV3 1HN<sub>N-P</sub> 2HN<sub>P-M</sub> 3918GU<sub>HN-L</sub>.

38. The chimeric PIV of claim 32, which contains protective antigens from one, two, three or four pathogens.

39. The chimeric PIV of claim 32, which contains protective antigens from one to four pathogens selected from HPIV3, HPIV1, HPIV2, and measles virus.

40. The chimeric PIV of claim 32, wherein said one or more supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to the wild-type HPIV3 genome length of 15,462 nt.

41. The chimeric PIV of claim 32, wherein the addition of said one or more supernumerary heterologous gene(s) or genome segment(s) specifies an attenuation phenotype of the chimeric PIV which exhibits at least a 10-to 100-fold decrease in replication in the upper and/or lower respiratory tract.

42. The chimeric PIV of claim 1, wherein the vector genome or antigenome is a human-bovine chimeric PIV genome or antigenome.

43. The chimeric PIV of claim 42, wherein the human-bovine chimeric vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses

1                    45.     The chimeric PIV of claim 44, wherein a transcription unit comprising  
2     an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector genome or  
3     antigenome for a corresponding N ORF of a HPIV3 vector genome.

1                    46.        The chimeric PIV of claim 45, wherein the vector genome or  
2        antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

1                    47.      The chimeric PIV of claim 48, which is rHPIV3-N<sub>B</sub> HA<sub>P-M</sub>.

1                    48.     The chimeric PIV of claim 42, wherein the vector genome or  
2 antigenome comprises a partial or complete BPIV genome or antigenome combined with one  
3 or more heterologous gene(s) or genome segment(s) from a HPIV.

49. The chimeric PIV of claim 48, wherein one or more HPIV gene(s) or genome segment(s) encoding HN and/or F glycoproteins or one or more immunogenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or complete bovine genome or antigenome to form the vector genome or antigenome.

1                    50.     The chimeric PIV of claim 49, wherein both HPIV3 genes encoding  
2     HN and F glycoproteins are substituted for corresponding BPIV3 HN and F genes to form  
3     the vector genome or antigenome.

1                    51.     The chimeric PIV of claim 50, wherein the vector genome or  
2     antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a  
3     supernumerary gene insert.

1                    52.     The chimeric PIV of claim 51, which is selected from rBHPIV3-G1 or  
2     rB/HPIV3-F1.

1                    53.     The chimeric PIV of claim 49, wherein one or more HPIV1 HN  
2     and/or F gene(s) or genome segment(s) encoding one or more immunogenic domain(s),  
3     fragment(s) or epitope(s) thereof are incorporated within the partial or complete bovine  
4     genome or antigenome to form the vector genome or antigenome, which is further modified

by incorporation of one or more HPIV2 HN and/or F gene(s) or genome segment(s) encoding one or more immunogenic domain(s), fragment(s) or epitope(s) thereof to form the chimeric genome or antigenome which expresses protective antigen(s) from both HPIV1 and HPIV2.

54. The chimeric PIV of claim 53, which is selected from rB/HPIV3.1-2F; rB/HPIV3.1-2HN; or rB/HPIV3.1-2F,2HN.

55. The chimeric PIV of claim 1, wherein the vector genome or antigenome is modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a heterologous PIV or non-PIV pathogen to form the chimeric genome or antigenome.

56. The chimeric PIV of claim 55, wherein the vector genome or antigenome is modified to encode a chimeric glycoprotein incorporating one or more antigenic domains, fragments, or epitopes from a second, antigenically distinct PIV to form the chimeric genome or antigenome.

57. The chimeric PIV of claim 55, wherein the chimeric genome or antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or epitopes from two or more HPIVs.

58. The chimeric PIV of claim 55, wherein the heterologous genome segment encodes a glycoprotein ectodomain which is substituted for a corresponding glycoprotein ectodomain in the vector genome or antigenome.

59. The chimeric PIV of claim 55, wherein one or more heterologous genome segment(s) of a second, antigenically distinct HPIV encoding said one or more antigenic domains, fragments, or epitopes is/are substituted within a HPIV vector genome or antigenome to encode said chimeric glycoprotein.

60. The chimeric PIV of claim 55, wherein heterologous genome segments encoding both a glycoprotein ectodomain and transmembrane region are substituted for counterpart glycoprotein ecto- and transmembrane domains in the vector genome or antigenome.



62. The chimeric PIV of claim 56, wherein the PIV vector genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct PIV is selected from HPIV1 or HPIV2.

1                    63.     The chimeric PIV of claim 62, wherein the HPIV vector genome or  
2     antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct  
3     HPIV is HPIV2.

64. The chimeric PIV of claim 63, wherein one or more glycoprotein  
ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein  
ectodomain(s) in the HPIV3 vector genome or antigenome.

1                    65.     The chimeric PIV of claim 64, wherein both glycoprotein  
2     ectodomain(s) of HPIV2 HN and F glycoproteins are substituted for corresponding HN and  
3     F glycoprotein ectodomains in the HPIV3 vector genome or antigenome.

1            66.     The chimeric PIV of claim 65, which is rPIV3-2TM.

1                    67.     The chimeric PIV of claim 55, which is further modified to  
2     incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3  
3     JS *cp45*.

1            68.     The chimeric PIV of claim 55, which is rPIV3-2TM*cp45*

69. The chimeric PIV of claim 55, wherein PIV2 ectodomain and transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more corresponding PIV3 cytoplasmic tail region(s).

1                    70.     The chimeric PIV of claim 69, wherein ectodomain and  
2     transmembrane regions of both PIV2 HN and F glycoproteins are fused to corresponding  
3     PIV3 HN and F cytoplasmic tail regions.

1            71.     The chimeric PIV of claim 70, which is rPIV3-2CT.

1                   72.     The chimeric PIV of claim 71, which is further modified to  
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3  
3 JS *cp45*.

1                   73.     The chimeric PIV of claim 72, which is rPIV3-2CT*cp45*.

1                   74.     The chimeric PIV of claim 55, which is further modified to  
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3  
3 JS *cp45* selected from mutations specifying an amino acid substitution in the L protein at a  
4 position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a  
5 position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position  
6 corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the  
7 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or  
8 a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS  
9 *cp45*

1                   75.     The chimeric PIV of claim 55, wherein a plurality of heterologous  
2 genes or genome segments encoding antigenic determinants of multiple heterologous PIVs  
3 are added to or incorporated within the partial or complete HPIV vector genome or  
4 antigenome.

1                   76.     The chimeric PIV of claim 75, wherein said plurality of heterologous  
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and  
3 are added to or incorporated within a partial or complete HPIV3 vector genome or  
4 antigenome.

1                   77.     The chimeric PIV of claim 55, wherein the chimeric PIV genome or  
2 antigenome is attenuated by addition or incorporation of one or more gene(s) or genome  
3 segment(s) from a bovine PIV3 (BPIV3).

1                   78.     The chimeric PIV of claim 55, wherein the chimeric genome or  
2 antigenome is modified by introduction of an attenuating mutation involving an amino acid  
3 substitution of phenylalanine at position 456 of the HPIV3 L protein.

1                   79.     The chimeric PIV of claim 78, wherein phenylalanine at position 456  
2 of the HPIV3 L protein is substituted by leucine.

1           80.    The chimeric PIV of claim 55, wherein the chimeric genome or  
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s) encoding  
3 one or more antigenic determinants from respiratory syncytial virus (RSV) or measles virus.

1           81.    The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome is modified by addition or substitution of one or more heterologous gene(s) or  
3 genome segment(s) that confer increased genetic stability or that alter attenuation,  
4 reactogenicity *in vivo*, or growth in culture of the chimeric virus.

1           82.    The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome is modified by introduction of one or more attenuating mutations identified in a  
3 biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA  
4 virus.

1           83.    The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 present within PIV3 JS *cp45*.

1           84.    The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 specifying an amino acid substitution in the L protein at a position corresponding to Tyr<sub>942</sub>,  
4 Leu<sub>992</sub>, or Thr<sub>1558</sub> of in JS *cp45*; in the N protein at a position corresponding to residues Val<sub>96</sub>  
5 or Ser<sub>389</sub> of JS *cp45*, in the C protein at a position corresponding to Ile<sub>96</sub> of JS *cp45*, in the F  
6 protein at a position corresponding to residues Ile<sub>420</sub> or Ala<sub>450</sub> of JS *cp45*, in the HN protein  
7 at a position corresponding to residue Val<sub>384</sub> of JS *cp45*, a nucleotide substitution a 3' leader  
8 sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of  
9 JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to  
10 nucleotide 62 of JS *cp45*.

1           85.    The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates attenuating mutations from different biologically derived mutant  
3 PIVs or other mutant nonsegmented negative stranded RNA virus.

1           86.    The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates an attenuating mutation at an amino acid position corresponding to

3 an amino acid position of an attenuating mutation identified in a heterologous, mutant  
4 negative stranded RNA virus.

1                    87.     The chimeric PIV of claim 86, wherein said attenuating mutation  
2     comprises an amino acid substitution of phenylalanine at position 456 of the HPIV3 L  
3     protein.

1                    88.     The chimeric PIV of claim 87, wherein phenylalanine at position 456  
2     of the HPIV3 L protein is substituted by leucine.

1                    89.     The chimeric PIV of claim 82, wherein the chimeric genome or  
2     antigenome includes at least one attenuating mutation stabilized by multiple nucleotide  
3     changes in a codon specifying the mutation.

1            90.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2     antigenome comprises an additional nucleotide modification specifying a phenotypic change  
3     selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-  
4     adaptation, plaque size, host-range restriction, or a change in immunogenicity.

1            91.     The chimeric PIV of claim 90, wherein the additional nucleotide  
2     modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes and/or a 3'  
3     leader, 5' trailer, and/or intergenic region within the vector genome or antigenome or within  
4     the heterologous gene(s) or gene segment(s).

1                    92.      The chimeric PIV of claim 91, wherein one or more PIV gene(s) is  
2      deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in  
3      an RNA editing site, by a frameshift mutation, by a mutation that alters an amino acid  
4      specified by an initiation codon, or by introduction of one or more stop codons in an open  
5      reading frame (ORF) of the gene.

1                    93.        The chimeric PIV of claim 92, wherein the additional nucleotide  
2        modification comprises a partial or complete deletion of one or more C, D or V ORF(s) or  
3        one or more nucleotide change(s) that reduces or ablates expression of said one or more C, D  
4        or V ORF(s).

1            94.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2     antigenome is further modified to encode a cytokine.



5 viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,  
6 filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 106. The method of claim 99, wherein the chimeric PIV elicits a  
2 polyspecific immune response against a human PIV (HPIV) and measles virus.

1 107. The method of claim 106, wherein the chimeric PIV elicits a  
2 polyspecific immune response against HPIV3 and measles virus.

1 108. The method of claim 99, wherein a first, chimeric PIV according to  
2 claim 1 and a second PIV are administered sequentially or simultaneously to elicit a  
3 polyspecific immune response.

1 109. The method of claim 108, wherein the second PIV is a second,  
2 chimeric PIV according to claim 1.

1 110. The method of claim 108, wherein the first, chimeric PIV and second  
2 PIV are administered simultaneously in a mixture.

1 111. The method of claim 108, wherein the first, chimeric PIV and second  
2 PIV are antigenically distinct variants of HPIV.

1 112. The method of claim 111, wherein the first, chimeric PIV comprises a  
2 partial or complete HPIV3 genome or antigenome combined with one or more heterologous  
3 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a different  
4 PIV.

1 113. The method of claim 111, wherein the first, chimeric PIV and second  
2 PIV each incorporate one or more heterologous gene(s) or genome segment(s) encoding one  
3 or more antigenic determinant(s) of a non-PIV pathogen.

1 114. The method of claim 113, wherein the first and second chimeric PIV  
2 incorporate one or more heterologous gene(s) or genome segment(s) encoding one or more  
3 antigenic determinant(s) of the same non-PIV pathogen.

1 115. A method for sequential immunization to stimulate the immune  
2 system of an individual to induce protection against multiple pathogens comprising  
3 administering to a newborn to 4 month old infant an immunologically sufficient amount of a

4 first attenuated chimeric HPIV expressing an antigenic determinant of a non-PIV pathogen  
5 and one or more antigenic determinants of HPIV3 and subsequently administering an  
6 immunologically sufficient amount of a second attenuated chimeric HPIV expressing an  
7 antigenic determinant of a non-PIV pathogen and one or more antigenic determinants of  
8 HPIV1 or HPIV2.

1 116. The method for sequential immunization of claim 115, wherein the  
2 first attenuated chimeric HPIV is an HPIV3 expressing a measles virus antigenic determinant  
3 and wherein the second attenuated chimeric HPIV is a PIV3-1 chimeric virus expressing a  
4 measles virus antigenic determinant and incorporating one or more attenuating mutations of  
5 HPIV3 JS *cp45*.

1 117. The method for sequential immunization of claim 115, wherein  
2 following the first vaccination, the vaccinee elicits a primary antibody response against both  
3 PIV3 and the non-PIV pathogen, but not HPIV1 or HPIV2, and upon secondary  
4 immunization the vaccinee is readily infected with the second attenuated HPIV and develops  
5 both a primary antibody response to HPIV1 or HPIV2 and a high titered secondary antibody  
6 response against the non-PIV pathogen.

1 118. The method for sequential immunization of claim 115, wherein the  
2 first chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV  
3 elicits an immune response against HPIV1 or HPIV2, and wherein both the first and second  
4 chimeric PIVs elicit an immune response against measles or RSV.

1 119. The method for sequential immunization of claim 115, wherein the  
2 non-PIV pathogen is selected from measles virus, subgroup A and subgroup B respiratory  
3 syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and type 2 human  
4 immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein  
5 Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 120. The method for sequential immunization of claim 115, wherein the  
2 second chimeric PIV comprises a partial or complete HPIV3 vector genome or antigenome  
3 combined with one or more gene(s) or genome segment(s) encoding one or more HPIV1  
4 and/or HPIV2 HN and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s)  
5 thereof.

1                    123.    The immunogenic composition of claim 122, formulated in a dose of  
2     $10^3$  to  $10^7$  PFU.

1                    125.    The immunogenic composition of claim 122, wherein the chimeric  
2    PIV elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2  
3    and HPIV3.

1                    127.    The immunogenic composition of claim 122, wherein the chimeric  
2    PIV elicits a polyspecific immune response against one or more HPPIVs and a heterologous  
3    pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial  
4    viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency  
5    viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,  
6    filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.



1                   128. The immunogenic composition of claim 127, wherein the chimeric  
2 PIV elicits a polyspecific immune response against HPIV3 and measles or respiratory  
3 syncytial virus

1                   129. The immunogenic composition of claim 122, further comprising a  
2 second, chimeric PIV according to claim 1.

1                   130. The immunogenic composition of claim 129, wherein the first and  
2 second chimeric PIVs are antigenically distinct variants of HPIV and bear the same or  
3 different heterologous antigenic determinant(s).

1                   131. The immunogenic composition of claim 129, wherein the first  
2 chimeric PIV comprises a partial or complete HPIV3 genome or antigenome combined with  
3 one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
4 determinant(s) of a non-PIV heterologous pathogen.

1                   132. The immunogenic composition of claim 129, wherein the second  
2 chimeric PIV incorporates one or more heterologous gene(s) or genome segment(s) encoding  
3 one or more antigenic determinant(s) of the same non-PIV heterologous pathogen.

1                   133. The immunogenic composition of claim 129, wherein the first  
2 chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits  
3 an immune response against HPIV1 or HPIV2, and wherein both the first and second  
4 chimeric PIVs elicit an immune response against the non-PIV pathogen.

1                   134. The immunogenic composition of claim 129, wherein the  
2 heterologous pathogen is selected from measles virus, subgroup A and subgroup B  
3 respiratory syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and  
4 type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies  
5 virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza  
6 viruses.

1                   135. The immunogenic composition of claim 129, wherein the  
2 heterologous pathogen is selected from measles virus or RSV.

1                   136. The immunogenic composition of claim 129, wherein the second  
2 chimeric PIV comprises a partial HPIV3 vector genome or antigenome combined with one



1 144. The isolated polynucleotide of claim 138, wherein the vector genome  
2 or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the  
3 heterologous pathogen is selected from measles virus, subgroup A and subgroup B  
4 respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2  
5 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,  
6 Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza  
7 viruses.

1                    145.    The isolated polynucleotide of claim 144, wherein said one or more  
2    heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins,  
3    subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps  
4    virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2 human  
5    immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC,  
6    gD, E, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus  
7    gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and NS1 proteins, and  
8    alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

1                    146.    The isolated polynucleotide of claim 138, wherein the vector genome  
2    or antigenome is a partial or complete HPIV3 genome or antigenome or a chimeric HPIV  
3    genome or antigenome comprising a partial or complete HPIV3 genome or antigenome  
4    having one or more gene(s) or genome segment(s) encoding one or more antigenic  
5    determinant(s) of a heterologous HPIV added or incorporated therein.

1 147. The isolated polynucleotide of claim 146, wherein the heterologous  
2 pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from  
3 the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1                    148.    The isolated polynucleotide of claim 147, wherein a transcription unit  
2    comprising an open reading frame (ORF) of a measles virus HA gene is added to or  
3    incorporated within a HPIV3 vector genome or antigenome.

1                    149.    The isolated polynucleotide of claim 147, wherein a transcription unit  
2    comprising an open reading frame (ORF) of a measles virus HA gene is added to or

1                    150.    The isolated polynucleotide of claim 138, wherein the partial or  
2    complete PIV vector genome or antigenome is combined with one or more supernumerary  
3    heterologous gene(s) or genome segment(s) to form the chimeric PIV genome or  
4    antigenome.

1                    151.    The isolated polynucleotide of claim 150, wherein the vector genome  
2    or antigenome is a partial or complete HPIV3 genome or antigenome and said one or more  
3    supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN,  
4    HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene  
5    unit.

1                    152.    The isolated polynucleotide of claim 138, wherein one, two or all of  
2    the HPIV1 HN, HPIV2 HN, and measles virus HA ORFs are added to the vector genome or  
3    antigenome.

1                    153. The isolated polynucleotide of claim 138, wherein one or more of the  
2    HPIV1 HN and HPIV2 HN genes and a 3918-nt GU insert is/are added are added to the  
3    vector genome or antigenome.

1                    154.    The isolated polynucleotide of claim 150, wherein said one or more  
2    supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign  
3    sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to  
4    the wild-type HPIV3 genome length of 15,462 nt.

1                    155.    The isolated polynucleotide of claim 138, wherein the vector genome  
2    or antigenome is a human-bovine chimeric PIV genome or antigenome.

1                    156.    The isolated polynucleotide of claim 155, wherein the human-bovine  
2    chimeric vector genome or antigenome is combined with one or more heterologous gene(s)  
3    or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous  
4    pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial  
5    viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency  
6    viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,  
7    filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses

1                    158.    The isolated polynucleotide of claim 157, wherein a transcription unit  
2    comprising an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector  
3    genome or antigenome for a corresponding N ORF of a HPIV3 vector genome.

1                    159.    The isolated polynucleotide of claim 158, wherein the vector genome  
2    or antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

1                    160.    The isolated polynucleotide of claim 138, wherein the vector genome  
2    or antigenome comprises a partial or complete BPIV genome or antigenome combined with  
3    one or more heterologous gene(s) or genome segment(s) from a HPIV.

1                    161. The isolated polynucleotide of claim 160, wherein one or more HPIV  
2 gene(s) or genome segment(s) encoding HN and/or F glycoproteins or one or more  
3 immunogenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated  
4 within the partial or complete bovine genome or antigenome to form the vector genome or  
5 antigenome.

1                    162.    The isolated polynucleotide of claim 161, wherein both HPIV3 genes  
2    encoding HN and F glycoproteins are substituted for corresponding BPIV3 HN and F genes  
3    to form the vector genome or antigenome.

1                    163.    The isolated polynucleotide of claim 162, wherein the vector genome  
2    or antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a  
3    supernumerary gene insert.

1                    164.    The isolated polynucleotide of claim 138, wherein the chimeric  
2    genome or antigenome encodes a chimeric glycoprotein having antigenic domains,  
3    fragments, or epitopes from both a human PIV (HPIV) and a heterologous pathogen.

1                    165.    The isolated polynucleotide of claim 164, wherein the chimeric  
2    genome or antigenome encodes a chimeric glycoprotein having antigenic domains,  
3    fragments, or epitopes from two or more different PIVs.



1 174. An expression vector comprising an operably linked transcriptional  
2 promoter, a polynucleotide sequence which includes a partial or complete PIV vector  
3 genome or antigenome of a human or bovine PIV combined with one or more heterologous  
4 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more  
5 heterologous pathogen(s) to form a chimeric PIV genome or antigenome, and a  
6 transcriptional terminator.

1                    176. The recombinant PIV of claim 175, wherein said polynucleotide insert  
2 is introduced into the PIV genome or antigenome in a reverse, non-sense orientation  
3 whereby the insert does not encode protein.

1                    178.    The recombinant PIV of claim 175, wherein said polynucleotide insert  
2    is approximately 3,000 nts or greater in length.

1            179.    The recombinant PIV of claim 175, wherein said recombinant PIV  
2    replicates efficiently *in vitro* and exhibits an attenuated phenotype *in vivo*.